WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 6.805
Volume 5, Issue 12, 865-879. Research Article ISSN 2277– 7105

CYPERMETHRIN INTOXICATES TESTICULAR BIOMARKERS THROUGH OXIDATIVE STRESS IN MATURE WISTAR MALE RAT AND ZINC ALLEVIATES IT

Tuhina Das, Anurag Paramanik, Rini Ghosh, Debjani Chatterjee, Sujata Maiti Choudhury*

Department of Human Physiology with Community Health, Vidyasagar University, Midnapore, West Bengal, India, Pin-721102.

Article Received on
23 Oct. 2016,

Revised on 13 Nov. 2016,
Accepted on 01 Dec. 2016

DOI: 10.20959/wjpr201612-7556

***Corresponding Author**
Sujata Maiti Choudhury
Department of Human Physiology with Community Health, Vidyasagar University, Midnapore, West Bengal, India, Pin-721102.

ABSTRACT

Cypermethrin, a synthetic pyrethroid pesticide, is used for more than one decade to control a wide variety of pests in agriculture. The present study was designed to investigate the toxic effects of cypermethrin on the male reproductive system of Wistar rats and also to assess the ameliorative role of zinc under these toxic conditions. Cypermethrin was administered alone at two dose levels and zinc was pretreated alone or combined with cypermethrin orally for 14 consecutivedays. Cypermethrin reduced the testicular index, prostatic index and epididymal index and sperm motility of rats in a dose dependent manner after the administration of cypermethrin. Significant increase in testicular malon-di-aldehyde (MDA) and GSSG levels were seen in the treated group whereas reduced glutathione (GSH) level of testis were decreased significantly in a dose-dependent manner compared to the control group. After the administration of cypermethrin, sperm motility, seminal plasma fructose concentration and testicular acid phosphatase were decreased significantly. Testicular glutamate pyruvate transaminase (GPT) and testicular glutamate oxaloacetate transaminase (GOT) levels were increased in cypermethrin intoxicated rats. The beneficial influences of zinc in attenuating the harmful effects of cypermethrin on male reproductive system of rat were observed in this study.

KEYWORDS: Cypermethrin; Zinc; Sperm motility; Seminal plasma fructose concentration Testicular acid phosphatase; Antioxidant parameters.

INTRODUCTION

Pesticides are used widely in agricultural substanceswhich are the largest group of poisonous substances. This is being disseminated throughout our environment in spite of their use in public health tocontrol insects, animals, weeds and disease vectors.[1,2] Increasing interest has been seen among health and environmental institutions regarding the potential reproductive effects due to exposure tothese agricultural and environmental chemicals.[3]

Exposure to pesticides causes reduction of sperm motility, producing abnormal sperm, decreased fertilizing ability in men and wildlife.[4] Pesticides have been shown tocause overproduction of reactive oxygen species (ROS) in both extra- and intracellular spaces, producing infertility in wild life and human.[5] The antioxidant parameters play an important role in protecting testes and thus inhibiting testicular dysfunction.[6]

Synthetic pyrethroid insecticides account for approximately one-fourth of worldwide insecticide market[7] and widely used in an increasing order during the last two decades with the declining use of organophosphate pesticides in agricultural fields, animal husbandry and indoor insect management. Pyrethroids are acutely toxic to fishes, than birds and mammal.[8]

Cypermethrin is an active synthetic pyrethroid insecticide is commonly used to control variouspests in agriculture, public health and in veterinary practice against ectoparasites.[9,10] It is chemically (+/-) alpha cyano-(3-phenoxyphenyl) methyl (+)-cis, trans-3-(2, 2-dichloroethenyl)-2, 2-dimethylcyclopropanecarboxylate.[10] It is highly toxic to bees, fishes, and water insects but has lower toxicity to birds. Ithas moderate carcinogenic, mutagenic, immunosuppressive and hepatotoxic effects. Low levels of cypermethrin in the aquatic environmentproduced a significant effect on mature male Atlantic salmon populations through disruption of reproductive functions.[11] Fenvalerate and cypermethrin were reported to impair reproductive functions of male rat inducing significant reductions in epididymal sperm count.[12] Cypermethrin altered mouse sperm head shape morphology as well asclastogenic effects on the root tip cells of *Allium cepa.* Besides these, decreased number of implantation sites and reduced number of viable fetuses in cypermethrin treated rabbits have been reported.[13] In dwarf goats, cypermethrin significantly (P<0.01) decreased the percentage of sperm motility, semen ejaculatory volume and concentration of spermatozoa.[14] Moreover, it is detected[15] that oedema between seminiferous tubules, vacuolation and hyalinization in the tubules of rat testis were caused by high doses of cypermethrin.

More recently, much attention hasbeen focused on the possible role of essential trace elements in providing the necessary preventive efficacy with less toxicity and side effects.[16,17,18] Zinc is a key constituent or cofactor of over 300 mammalian proteins. It is intensively being studied for its protective efficacy in various models of animal toxicity. Zinc is a beneficial agent in reducing the damage arising in increased oxidative stress.[19,20,18] Based on the above facts, the present study was conducted to investigate the effects of zinc in the amelioration of the damage imposed on the reproductive system of the rats intoxicated with cypermethrin.

MATERIALS AND METHODS

Chemicals and reagents

Cypermethrin 10% Emulsifiable Concentrate (EC) commercial name (Ustad), indole, sodiumhydroxide (NaOH), dinitrophenyl hydrazine (DNPH), thio-barbutaric acid (TBA), trichloroacetic acid (TCA), sodium chloride (NaCl), hydrochloric acid (HCl), sulfosalicyclic acid(SSA), 2,4,4-dithionitrobenzoic acid (DTNB),Tris HCl, potassium dihydrogen phosphate (KH2PO4), zinc sulphate (ZnSO4) were used in the present study. All chemicals used were analytical grade and obtained fromMerck Ltd., SRL Pvt. Ltd., Mumbai, India.

Animal care and treatment

The study was designed on 36 mature albino male Wistar rats weighing 130-150 gm. The animals were acclimatized for at least 1 week at an ambient temperature of 25°±2°C with 12 hrs light-dark cycle before prior to different treatments. The animals were fed on the standard pellet and water *ad libitum*, throughout the period of experiment. Thirty six albino mature male ratswere divided into equal six groups.Each group contains sixanimals. The groups weredesigned as: **Group I**: Control (5 ml /kg body wt.), **Group II**: Zinc (227 mg/l in drinking water) control, **Group III**: Cypermethrin-treated (Low dose, 40mg/body wt.) group **Group IV**: Zinc + Cypermethrin-treated (Low dose, 40mg/body wt.) group, **Group V**: Cypermethrin-treated (High dose,80mg/body wt.) group and **Group VI**: Zinc + Cypermethrin-treated (High dose, 80mg/body wt.) group.

After one hour of the zinc administration in the form of $ZnSO_4.7H_2O$ (227 mg/l in drinking water[21], cypermethrin at the dose levels of 40 and 80mg /kg body weight were treated orally for 14 consecutive days. Animal's weight was taken daily and the dose was adjusted accordingly.On 15th day, the animals were sacrificed by cervical dislocation and samples were collected for the experiment.

Sample collection

Total body weights of rats in each group were taken before the treatment period and before sacrifice. 24 hrs after the last dose all rats were sacrificed by rapid decapitation. Blood was collected using EDTA as anticoagulant from the animals and serum was separated andkept at -20°C for biochemical studies. The testesand other male accessory sex organs (epididymis and prostate) were removed immediately and adhering fats were cleaned. Then testicular weights were recorded and stored properly for the biochemical estimation. Then, epididymis and prostate prepared for fertility evaluation and testicular tissues were taken for the determination of oxidative biomarkers.

Estimation of testicular and other male accessory sex organs indices

Testes and other male accessory sex organs of sacrificed male albino rat (Wister) were separated from its body and weights were taken after removing all fats from the organs. Organ indices were measured by the following formula:

Organ index = Organ weight/Body weight x 100.

Assay of epididymal sperm motility[22]

Sperm motility was assessed by the method of **WHO** and expressed as the percent motility.

Measurement of seminal fructose concentration[23]

Seminal plasma fructose concentration was measured by mixing 0.5 ml of tissue homogenate (20mg/ml, as sample), 0.5 ml of fructose (0.14 mM and 0.28 mM fructose, as two standards) and 0.5 ml of distilled water (as blank) with 0.5 ml of indole reagent separately. Then 5 ml of concentrated HCl was added to it. All test tubes were covered with cottonand incubated for 20 minat 50°c. All test tubes were cooled in icewater and then in room temperature. The reading was taken at 470 nm.

Testicular glutamate pyruvate transaminase (GPT) and testicular glutamate oxaloacetate transaminase (GOT)[24]

For the estimation of GPT, to 1.0 ml of the buffered substrate (200 mM/L DL-alanine and 2 mM/L of α- ketoglutarate pH=7.4), 0.1 ml of homogenate was added and incubated at 37°C for 30 minutes. The reaction was arrested by the addition of 1.0 ml of dinitrophenyl hydrazine and left aside for 20 minutes at room temperature. The colour developed by the addition of 10 ml of 0.4N sodium hydroxide was read at 505-540 nm in a spectrophotometer

(UV-Shimadzu-245, Japan) against the reagent blank. The enzyme activity in serum was expressed as IU/lit.

For the estimation of GOT, to 1.0 ml of the buffered substrate (200 mM/L of DL-aspartate and 2 mM/L of α-ketoglutarate, pH=7.4), 0.1 ml of homogenate was added and incubated for one hour at 37°C. Then, 1.0 ml of the dinitrophenyl hydrazine reagent was added and left for 20 minutes. At the end of 20 minutes, 10 ml of 0.4N sodium hydroxide was added and the color developed was read at 505-540 nm in a spectrophotometer (UV-Shimadzu-245, Japan) after 10 minutes. The standards were also treated similarly. The enzyme activity in serum was expressed as IU/lit.

Assay of acid phosphatase[25]

The acid phosphatase activity of testis was measured using *p*-nitrophenol phosphate as a substrate. Amount of PNP liberated was measured at 420 nm by spectrophotometer (UV-245 Shimadzu, Japan).

Estimation of oxidative stress parameters

Testicular Malondialdehyde[26]

One ml homogenate (20 mg tissue/ 1 ml of 0.05 mM PBS) was mixed with 0.2 ml of 8.1% sodium dodecyl sulfate, 1.5 ml of acetate buffer (20% pH 3.5) and 1.5 ml of aqueous solution of thiobarbituric acid (0.8%). After heating at 95°C for 60 min, produced red pigment was extracted with 5 ml of *n*-butanol-pyridine mixture (15: 1) and was centrifuged at 5000 rpm for 10 min at room temperature. The absorbance of supernatants was noted at 535nm.

Testicular Reduced Glutathione[27]

200µl of tissue homogenate (20mg/ml) was mixed with 100 µl of sulfosalicylic acid and the mixture was centrifuged for 10 min at 3000 rpm. Then 200µl supernatant was added to 1.8 ml of DTNB and mixed well. Then the reading was taken at 412-420nm.

Testicular Oxidised Glutathione[28]

Oxidized glutathione of testis was assayed by the method of Griffith. 100µl of sample was mixed with 2-vinyl pyridine and incubated for 1hr at 37˚C. Then sulfosalicylic acid (4g%) was added to it and mixed well. It was kept in room temperature for 30min. and centrifuged at 2000 rpm for 10 min. Then 200 µl of supernatant was mixed with DTNB (4mg %) and the reading was taken at 412 nm.

Statistical analysis

The results were expressed as the Mean ± Standard error of mean (SEM). Statistical analysis of the collected data were performed by Analysis of variance (ANOVA) followed by multiple comparison two-tail t-test. The difference was considered significant when $p<0.05$.

RESULTS

Table 1 shows the effect of zinc on testiculo-somatic index, prostatic index and epididymal index in cypermethrin induced male albino rats. The organ indices has been decreased significantly ($p<0.001$) in cypermethrin treated groups which elevated by the treatment of zinc.

The effect of zinc on rat epididymal sperm motility in cypermethrin induced male albino rat is shown in Table 2. In cypermethrin treated groups, epididymal sperm motility decreased significantly ($p<0.001$) in the dose dependent manner compared to the control group. Treatment of zinc diminished the cypermethrin toxicity and restored the normal status of the testicular tissue to a great extent.

Figure 1 shows the effect of zinc on the seminal plasma fructose concentration of cypermethrin induced male albino rat. Seminal vesicular fructose levels were significantly lower in cypermethrin treated groups compared to the control animals. Zinc inhibit the toxicity.

Testicular glutamate pyruvate transaminase (GPT) (Figure 2) and testicular glutamate oxaloacetate transaminase (GOT) (Figure-3) levels were increased in cypermethrin intoxicated rats in a dose dependent manner which were altered after the treatment of zinc.

In figure 4, therewas a significant changein the testicular ACP in comparison to the control. Significant decrease ($P<0.001$) was found in cypermethrin induced group where treatment with zinc ameliorated the toxicity.

Figure 5 shows the effect of zinc on testicular malon-di-aldehyde (MDA) in cypermethrin induced male albino rat. The testicular MDA level increased significantly ($p<0.05$, $p<0.001$) in cypermethrin-intoxicated groups in a dose dependent manner.

Figure 6 and 7 shows the effect of zinc on reduced and oxidized glutathione in cypermethrin treated male albino rat respectively. The testicular GSH was decreased significantly

($p<0.001$) whereas GSSG was increased in the dose dependent manner compared to the control group and zinc ameliorated the effects.

Table 1 shows the effect of zinc on testicular, prostatic and Adrenal index in cypermethrin induced male rats

Group	Testiculo-somatic index	Prostatic index	Adrenal index
Group-I Control (5 ml /kg body wt)	1.96±0.017	0.293±0.006	0.113±0.01
Group-II Zinc control(227 mg/l)	2.44±0.016	0.313±0.006	0.114±0.01
Group-III Cypermethrin low dose (40 mg/ kg body wt.)	1.74±0.014 a***	0.206±0.007 a**	0.045±0.001 a***
Group-IV Zinc(227 mg/l) + cypermethrin low dose (40 mg/ kg body wt.)	1.84±0.012 a***b**	0.261±0.007 a* b*	0.071±0.002 a***b**
Group-V Cypermethrin high dose (80 mg/ kg body wt.)	0.64±0.015 a***	0.068±0.004 a***	0.036±0.003 a***
Group-VI Zinc (227 mg/l) + cypermethrin high dose (80 mg/ kg body wt.)	1.36±0.074a**c** *	0.18±0.007 a*** c**	0.124±0.07 a* c***

Results are expressed as Mean±SEM. Analysis is done by ANOVA followed by multiple comparison two-tail t-tests. Superscript a Group I versus all other groups; superscript b Group III versus Group IV and superscript c Group V versus Group VI (*indicates $p<0.05$, ** indicates $p<0.01$ and *** represents $p<0.001$).

Table 2 shows the effect of Zinc on sperm motility in Cypermethrin induced male rats

Group	Sperm motiliy
Group-I Control (5 ml /kg body wt)	64.33±0.98
Group-II Zinc control (227 mg/l)	68.33±0.88
Group-III Cypermethrin low dose (40 mg/ kg body wt.)	48.83±0.66 a***
Group-IV Zinc(227 mg/l) + cypermethrin low dose (40 mg/ kg body wt.)	57.66±0.7 a***b***
Group-V Cypermethrin high dose (80 mg/ kg body wt.)	40.16±0.57 a***
Group-VI Zinc (227 mg/l) + cypermethrin high dose (80 mg/ kg body wt.)	50±0.57 a***c***

Results are expressed as Mean±SEM. Analysis is done by ANOVA followed by multiple comparison two-tail t-tests. Superscript a Group I versus all other groups; superscript b Group III versus Group IV and superscript c Group V versus Group VI (*** represents p<0.001).

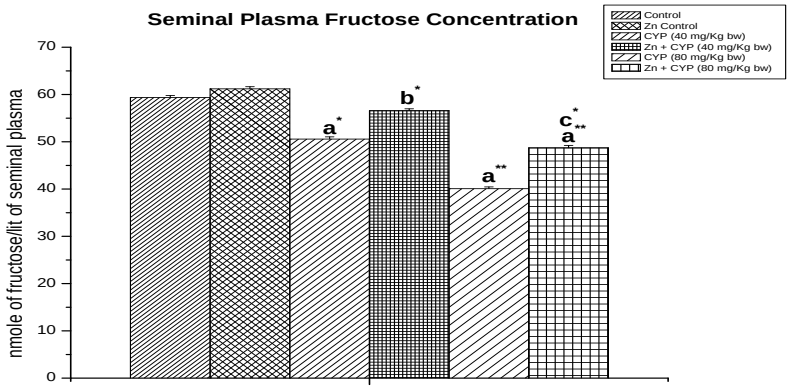

Figure-1 shows the effect of zinc on seminal plasma fructose concentration in cypermethrin induced male albino rats. Results are expressed as Mean±SEM. Analysis is done by ANOVA followed by multiple comparison two-tail t-tests. Superscript a Group I versus all other groups; superscript b Group III versus Group IV and superscript c Group V versus Group VI (*indicates p<0.05, ** indicates p<0.01)

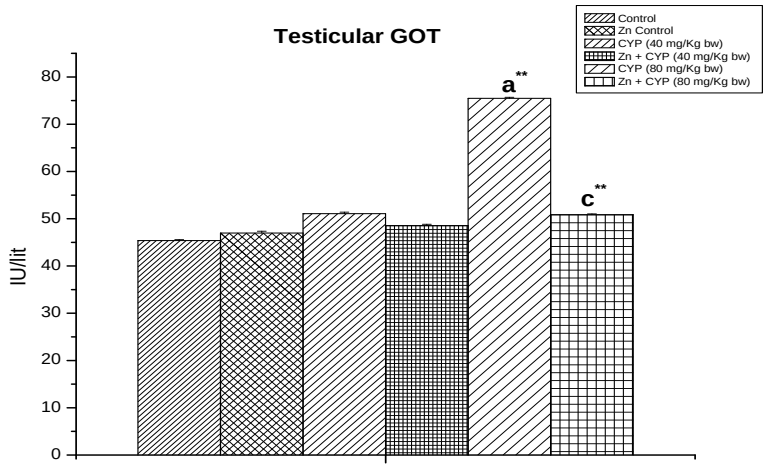

Figure-2 shows the effect of zinc on testicular GOT activity in cypermethrin induced male albino rats. Results are expressed as Mean±SEM. Analysis is done by ANOVA followed by multiple comparison two-tail t-tests. Superscript a Group I versus all other groups; superscript b Group III versus Group IV and superscript c Group V versus Group VI (indicates p<0.01)**

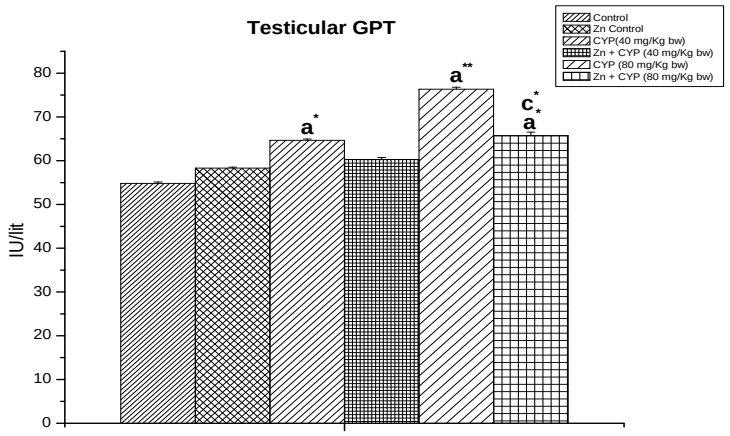

Figure-3 shows the effect of zinc on testicular GPT activity in cypermethrin induced male albino rats. Results are expressed as Mean±SEM. Analysis is done by ANOVA followed by multiple comparison two-tail t-tests. Superscript a Group I versus all other groups; superscript b Group III versus Group IV and superscript c Group V versus Group VI (*indicates p<0.05, ** indicates p<0.01)

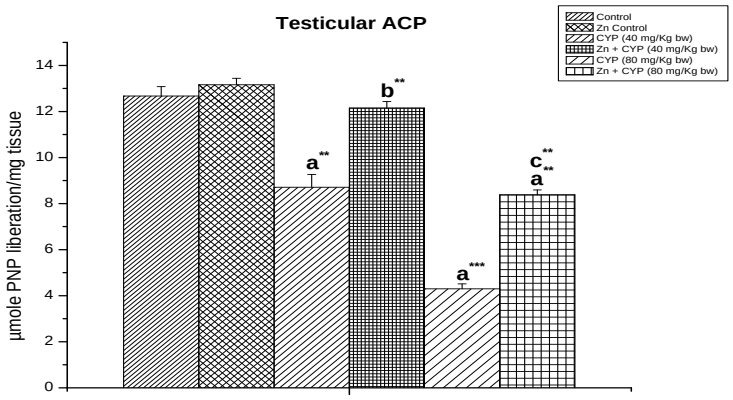

Figure-4 shows the effect of zinc on testicular ACP activity in cypermethrin induced male albino rats. Results are expressed as Mean±SEM. Analysis is done by ANOVA followed by multiple comparison two-tail t-tests. Superscript a Group I versus all other groups; superscript b Group III versus Group IV and superscript c Group V versus Group VI (indicates p<0.01 and *** represents p<0.001)**

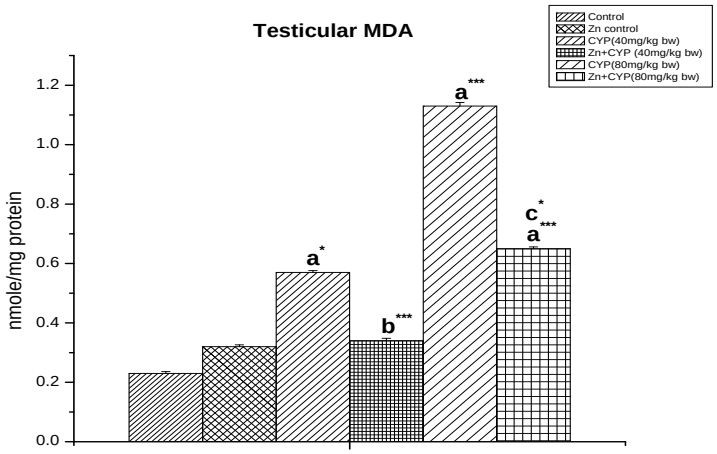

Figure-5 shows the effect of zinc on testicular MDA level in cypermethrin induced male albino rats. Results are expressed as Mean±SEM. Analysis is done by ANOVA followed by multiple comparison two-tail t-tests. Superscript a Group I versus all other groups; superscript b Group III versus Group IV and superscript c Group V versus Group VI (*indicates p<0.05, and * represents p<0.001)**

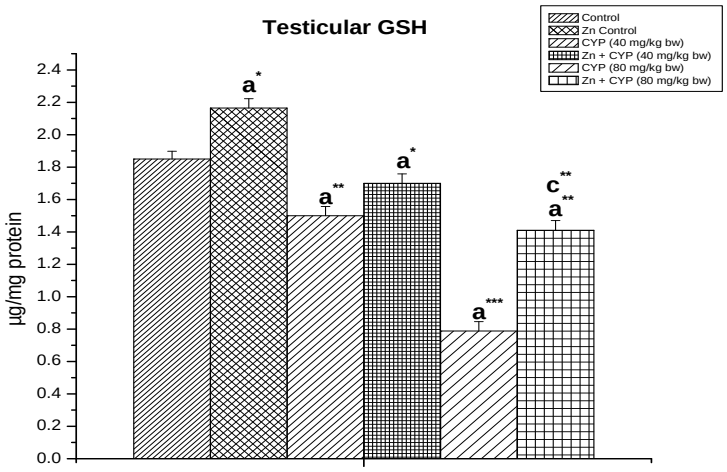

Figure-6 shows the effect of zinc on testicular GSH content in cypermethrin induced male albino rats. Results are expressed as Mean±SEM. Analysis is done by ANOVA followed by multiple comparison two-tail t-tests. Superscript a Group I versus all other groups; superscript b Group III versus Group IV and superscript c Group V versus Group VI (*indicates p<0.05, ** indicates p<0.01 and * represents p<0.001)**

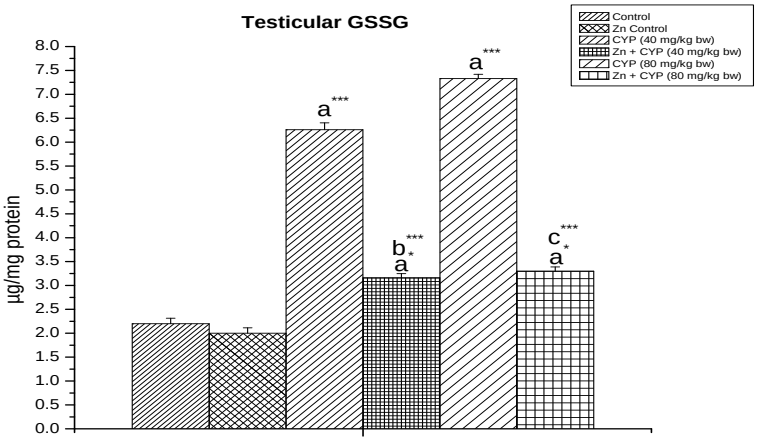

Figure-7 shows the effect of zinc on testicular GSSG content in cypermethrin induced male albino rats. Results are expressed as Mean±SEM. Analysis is done by ANOVA followed by multiple comparison two-tail t-tests. Superscript a Group I versus all other groups; superscript b Group III versus Group IV and superscript c Group V versus Group VI (*indicates p<0.05, and * represents p<0.001)**

DISCUSSION

The present study was evaluated the adverse effects of cypermethrin on the reproductive system of male Wistar rats and also to assess the preventive role of zinc under this toxic condition. Organ weight is basic parameters for the toxicological studies.[29] The testicular weight is generally dependent on the mass of the differentiated spermatogenic cells. Decreased number of germ cells, inhibition of spermatogenesis and steroidogenic enzyme activity may be the causes of reduction in testicular weight.[30] Furthermore, in the current study, cypermethrin decreased the weight of accessory male reproductive organs like epididymis and prostate in rat. It may be due to cypermethrin acted directly on the testes and affected the androgen biosynthesis in Leydig cells; this decrease in testosterone attenuated the function of steroid-sensitive organs, thus reducing their weight.[31] The testicular index was decreased by cypermethrin effectively elevated by the pretreatment of zinc. This may be due to the preventive role of zinc on testicular damage. It was reported[12] that cypermethrin decreased sperm motility in vitro. In this study, reduced sperm motility decreased may be due to alteration inmitochondrial enzyme activity of the spermatozoa, fructose synthesis and secretion by the accessory glands.

The reduction of fructose content in seminal fluid collected from cypermethrin intoxicated rats were draw attention towards the reduced secretary ability of seminal vesicles and the nutritive potential for the semen. Testicular inhibitory action generally seen by the elevation of testicular cholesterol level resulted from the inhibition in the testicular androgenesis.[32]

In testis increased testicular GPT and GOT levels suggest that cypermethrin causes testicular damage. Increase of transaminase activity along with the decreased of content of free radical (O2**.**) scavengers are probably the consequence of cypermethrin induced pathological changes in testis. In the present work, it was noticed that, there is a significant reduced ($p<0.001$) in the activities of a measured testicular enzymes ACP. This finding may be return to the damaged tubules and leydig cells due to the effect of cypermethrin on the composition of amino acids making up the cell plasma membrane proteins, leading to leakage of their contents to the outside of cells.[33]

Oxidative stress defines an imbalance between the formation of reactive oxygen species (ROS) and antioxidative defense mechanisms. During pyrethroid metabolism, reactive oxygen species (ROS) were generated and caused oxidative stress in intoxicated animals.[34] In oxidative stress, lipid peroxidation is occurred due to excessive free radical production and is considered a primary mechanism of cell membrane destruction and cell damage. Malon-di-aldehyde (MDA) is the end product of lipid peroxidation. Cypermethrin increased the testicular MDA and GSSG. Simultaneously it decreased testicular GSH content butzinc decreased the levels of lipid peroxidation (MDA) in male Wistar rats. GSH plays a major role in protecting the cell against oxidative damage by reacting with ROS. In healthy human cells normally ~98% of the total GSH exists in the reduced form while a much smaller fraction (~1%) exists in the oxidized forms – GSSG.[27,35,36] The present study demonstrated that zinc decreased the lipid peroxidation and thereby augmented the endogenous antioxidant enzyme in cypermethrin induced male rats and the observed properties may be attributed to the antioxidant properties present in zinc, which is one of the most essential properties of any pesticide antidote. The decreased GSH content and increased MDA and GSSG levels in testis suggest that cypermethrin caused testicular damage. Zinc used to prevent oxidative damage by interrupting the propagation of the oxidation of polyunsaturated fatty acids. Zinc is not distributed uniformly in tissues; it can show a protective effect especially when administered prior to cypermethrin.[37]

CONCLUSION

From the above discussion it can be concluded that cypermethrin-induced testicular toxicity, lipid peroxidation, oxidative stress in male rats and treatment with zinc has resulted in pronounced ameliorating effect especially on cypermethrin-induced male reproductive toxicity.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

ACKNOWLEDGEMENT

The authors are thankful to Vidyasagar University, Midnapore for providing laboratory facilities to execute these studies.

REFERENCES

1. Ecobichon DJ. Introduction in Pesticides and Neurological Diseases. CRC Press. 1994; 1-23.
2. Eddleston M., Karalliedde L. and Buckley N., Pesticide poisoning in the developing world-a minimum pesticides list, Lancet., 2002; 360: 1163-1167.
3. Dalsenter PR., Faqi AS. and Chahoud I., Serum testosterone and sexual behavior in rats after prenatal exposure to lindane, Bull Environ Contam Toxicol., 1997; 59: 360-366.
4. Alm H., Tiemann U. and Torner H., Influence of organochlorine pesticides on development of mouse embryos *in vitro*, Reprod Toxicol., 1996; 10: 321-326.
5. Sharpe RM. and Skakkebaek NE., Are oestrogens involved in falling sperm counts and disorders of the male reproductive tract?. The Lancet., 1993; 341: 1392-1395.
6. Oschsendorf FR., Infections in the male genital tract and reactive oxygen species, Human Reproduction Update., 1999; 5: 399- 420.
7. Casida JE. and Quistad GB., Golden age of insecticide research: Past, Present or Future?. Annu Rev Entomol., 1998; 43: 1-16.
8. Bateman DN., Management of pyrethroid exposure, J Toxicol Clin Toxicol., 2000; 38: 107-109.
9. World Health Organization: Cypermethrin. Environmental Health Criteria 82. WHO, Geneva, 1989.
10. Bhunya SP. and Pati PC., Genotoxic effects of synthetic pyrethroid insecticide Cypermethrin in mice *in vivo*, Toxicol Lett., 1988; 41(3): 223-230.

11. Moore A. and Waring CP., The effects of a synthetic pyrethroid pesticide on some aspects of reproduction in Atlantic salmon (*Salmo salar* L.), Aquat Toxicol., 2001; 52: 1– 12.
12. Song L., Wang YB., Sun H., Yuan C., Hong X., Qu JH., Zhou JW., Wang X., Effects of fenvalerate and cypermethrin on rat sperm motility patterns in vitro as measured by computer-assisted sperm analysis, J Toxicol Environ Health A., 2008; 71(5): 325-332.
13. Elbetieha A., Daas SI., Khamas W., Darmani H., Evaluation of the toxic potential of cypermethrin pesticide on some reproductive and fertility parameters in the male rats, Arch Environ Contam Toxicol., 2001; 41: 522-528.
14. Ahmad M., Hussain I., Khan A., Rehman N., Deleterious effects of cypermethrin on semen characteristics and testes of dwarf goats (*Capra hircus*), Experimental and Toxicologic Pathology, 2009; 61(4): 339-346.
15. Manna S., Bhattachary D., Mandal TK., Das S., Repeated dose toxicity of alphacypermethrin in rats, J Vet Sci., 2004; 5(3): 241-245.
16. Xiu YM., Trace elements in health and diseases, Biomed Environ Sci., 1996; 9: 130–136.
17. Kang YJ. and Zhou Z., Zinc prevention and treatment of alcoholic liver disease, Mol Aspects Med., 2005; 26: 391–404.
18. Zhou Z., Wang L., Song Z., Saari JT., McClain CJ., Kang YJ., Zinc supplementation prevents alcoholic liver injury in mice through attenuation of oxidative stress, American Journal of Pathology., 2005; 166(6): 1681–1690.
19. Cagen SZ. and Klaassen CD., Protection of carbon tetrachloride- induced hepatotoxicity by zinc: role of metallothionein, Toxicol Appl Pharmacol., 1979; 51: 107–116.
20. Cabre M., Ferre N., Folch J., Paternain JL., Hernandez M., del Castillo D., Joven J., Camps J., Inhibition of hepatic cell nucleus DNA fragmentation by zinc in carbon tetrachloride treated rats, J Hepatol., 1999; 31: 228–234.
21. Goel, 2006.
22. WHO laboratory manual for the examination of human semen and sperm–cervical mucus interaction. Fourth edition. New York. Cambridge University Press. 1999.
23. Karvonen MJ. and Malm M., Colorimetric Determination of Fructose with Indol, Scandinavian Journal of Clinical and Laboratory Investigation.,1955; 7(4): 305-7.
24. Goel BK. Routine biochemical test. In: Mukherjee KL, editor. Medical Laboratory Technology. III. New Delhi: Tata McGraw-Hill Publishing Company Ltd; 1988; 985–1079.

25. Vanha-Perttula T. and Nikkanen V. Acid phosphatases of the rat testis in experimental conditions, Acta Endocrinol., 1973; 72: 376–390.
26. Ohkawa H., Onishi N. and Yagi K., (1979). Assay for lipid per-oxidation in animal tissue by thiobarbituric acid reaction, Anal. Biochem., 1979; 95: 351-358.
27. Griffith OW., Glutathione turnover in human erythrocytes, J. Biochem., 1981; 256: 4900-4904.
28. Griffith OW., Determination of glutathione and glutathione disulphide using glutathione reductase and 2-vinylpyridine, Anal. Biochem., 1980; 106: 207-12.
29. Takahashi O. and Oishi S., Testicular toxicity of dietary 2,2-bis(4-hydroxyphenyl) propane (bisphenol A) in F344 rat, Arch Toxicol., 2001; 75: 42–51.
30. Yavasoglu A., Karaaslan MA., Uyanikgil Y., Sayim F., Ates U., Yavasoglu NU., Toxic effects of anatoxin-a on testes and sperm counts of male mice, Exp Toxicol Pathol., 2008; 60: 391–396.
31. Wang XZ., Liu SS., Sun Y., Wu JY., Zhou YL., Zhang JH., Beta-cypermethrin impairs reproductive function in male mice by inducing oxidative stress, Theriogenology., 2009; 72: 599–611.
32. Morris MD. and Chaikoff IL., The origin of cholesterol in liver, small intestine, adrenal gland and testis of the rat: dietary versus endogenous contribution, J Biol Chem., 1959; 234: 1095-1097.
33. Shaikh TM Al. and Elfayoumi RI., Protective effects of Vitamine E against testicular enzymes toxicity induced by Cypermethrin in mice, International Journal of Pharma and Bio Sciences., 2013; 4(2): (B) 942 – 953.
34. Gupta A., Nigam D., Gupta A., Shukla GS., Agarwal AK., Effect of pyrethroid based liquid mosquits repellent inhalation on the blood-brain barrier function and oxidative damage in selected organs of developing rats, J Appl Toxicol., 1999; 19: 67-72.
35. Dringen R. and Hirrlinger J., Glutathione pathways in the brain. Biol Chem., 2003; 384: 505–516.
36. Kennett E., Bubb W., Bansal P., Alewood P., Kuchel P., NMR studies of exchange between intra- and extracellular glutathione in human erythrocytes, Redox Report., 2005; 10(2): 83-90.
37. Packer L., Witt EH. and Tritschler HJ., Alpha- Lipoic acid as a biological antioxidant, Free Radic Biol Med., 1995; 19: 227–250.